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THE EFFECTS OF SALSOLINOL ON THE MUCOSAL MAST CELLS IN THE RAT GUT

Abstract: *The effects of salsolinol on the mucosal mast cells in the rat gut*

Mast cells in the gastrointestinal tract have been found in close spatial contact with the regulatory cells of gastrointestinal motility: interstitial cells of Cajal (ICC) and myenteric neurons, suggesting their functional interaction. Because of the regulatory role of mast cells even the slight damage or change in activity of these cells may cause considerable disorder of the gut motility. The catechol isoquinoline derivatives are endogenous compounds present in the mammalian brain and the representative one is referred to as salsolinol. Increased salsolinol levels are detected in the cerebrospinal fluid of Parkinson's disease patients. Gastrointestinal dysmotility in those patients has been associated with peripheral action of salsolinol. The aim of this study was to evaluate effects of exogenous salsolinol on mast cells in the gastrointestinal tract of rats.

Male Wistar rats (n = 8) were injected intraperitoneally with salsolinol (50 mg/kg/day) for 3 weeks and the equal group served as a control. On the last day the animals were sacrificed, stomachs, small and large intestines were removed, and paraffin embedded specimens were prepared. Slides were toluidine blue stained and the total number and percentage of degranulated mast cells in gastric antral, duodenal and ascending colon wall were assessed by image analysis.

The number of mast cells in the gastrointestinal wall was decreased in the salsolinol group compared to the control — in the stomach 98.7 ± 53.3 vs. 156.7 ± 45.8, in the duodenum 2.6 ± 2.1 vs. 7.83 ± 7.8 and in colon 12.8 ± 14 vs.10.7 ± 17.1 (salsolinol treated vs. control group). Carried out examinations showed the destructive action of salsolinol on the mast cells in all segments examined of gastrointestinal tract.

Key words: mast cells, salsolinol, rat, stomach, duodenum, colon
Słowa kluczowe: komórki tuczne, salsolinol, szczur, żołądek, dwunastnica, jelito grube

INTRODUCTION

The most diverse collection of nerves outside the central nervous system and the most extensive immune system in the body are hidden in the gastrointestinal system. Exposure to luminal factors, such as environmental irritants and infectious agents, triggers adaptive processes serving to restore homeostasis, where both neuroendocrine and immune responses are involved [1]. The neural apparatus of the gut is composed of a large number of enteric neurones arranged in networks of enteric ganglia, located mainly in the submucosal and myenteric plexus, and connected by interganglionic strands [2]. The enteric nervous system (ENS) stores a library of programs that generate specific patterns of stereotyped motor behaviour. The enteric immune system cooperates with the ENS to establish a first line defence. Several cell types, including lymphocytes, macrophages, dendrocytes and mast cells can be found in close anatomic association with enteric neurons, vagal nerves fibres and spinal sensory nerves. However, the most is known about enteric mast cells and enteric neurons interplay [3].

Communication between the central nervous system (CNS) and mast cells in the gut mucosa may occur through various pathways, of which the most important occurs at a local level, through intrinsic reflexes. Both mast cells and sensory neurons code information by releasing chemical message that is decoded by processing circuits in the nervous system. At the same time, enteric mast cells are used by the central nervous system as a link for sending chemical signals to the ENS [3, 4]. Reports of Pavlovian conditioning of enteric mast cell degranulation gives direct evidence for a brain-mast cell connection [5]. Therefore, mast cells participate in the modulation of a wide variety of gastrointestinal (GI) physiological and pathological processes.

Epidemiological evidence has revealed that gastrointestinal dysfunctions may constitute the initial clinical feature of Parkinson's disease (PD) and occur long before typical PD symptoms [6]. Neuroactive substances, which may induce the disease, are suggested to enter the body via the gastrointestinal tract, and attack the nervous system via enteric plexuses and preganglionic vagal fibres [7]. Catechol isoquinoline derivatives are endogenous compounds proposed as candidates of dopaminergic neurotoxins. The representative one is referred to as salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline), which can be endogenously synthesized from dopamine and acetaldehyde by salsolinol synthase or, alternatively, from dopamine and pyruvic acid [8]. Properties of salsolinol, as a neurotoxin eliciting symptoms almost identical to idiopathic Parkinson's disease, are intensively studied [9].

The aim of this study was to evaluate the influence of exogenous salsolinol on mucosal mast cells in the gastrointestinal tract of Wistar rats.

MATERIALS AND METHODS

Sixteen male Wistar rats (Poland) weighting 200 ± 20g were used for the study. The animals were housed individually in cages at constant temperature 22–24°C and on a 12 : 12 hour light: dark schedule. Solid chow and tap water were accessible ad libitum. The rats were randomly divided into 2 equal groups. One group (n = 8) was subjected to intraperitoneal injections with salsolinol (50 mg/kg/day; Sigma, USA) for 21 days and the other group (n = 8) served as the control. The experimental protocol was approved by the Jagiellonian University Bioethical Committee.

On the last day of the experiment animals were sacrificed, and stomachs, small and large intestines were removed. All tissue fragments were formalin fixed and paraffin-embedded 5 μm slices were prepared. Specimens were toluidine blue stained for mast cells assessment.

Images were collected using light microscope Axiophot (Zeiss) equipped with colour camcorder ProgRes C12 plus (Jenoptik) and quantified using Multiscan 18.03 Software (CSS, Warszawa). The number and percentage of degranulated

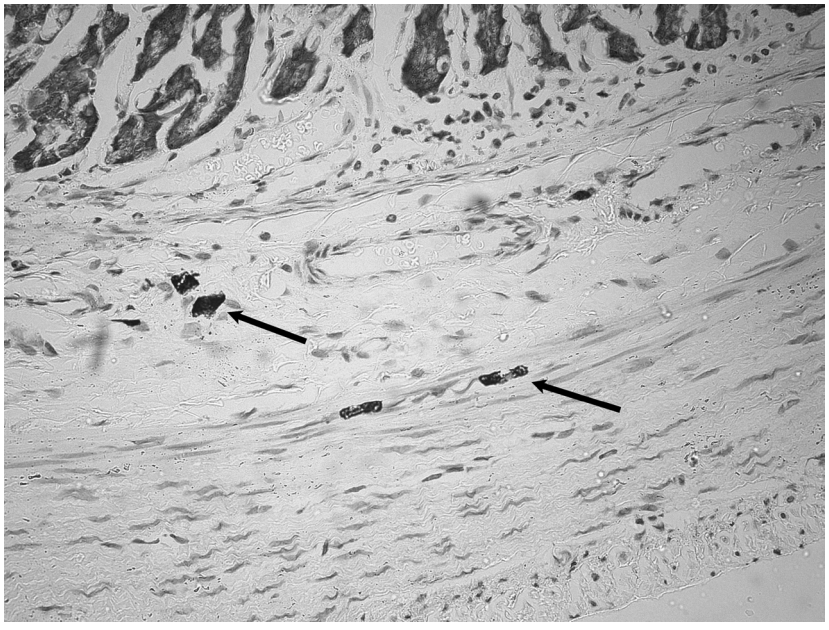

Fig. 1. Mast cells in the rat stomach wall (cross-section). Toluidine blue staining. Mast cells red-purple (metachromatic staining) and the background blue (orthochromatic staining). Some cells degranulating (arrows). Magnification 400 ×

Ryc. 1. Komórki tuczne w ścianie żołądka szczura (przekrój poprzeczny). Barwienie błękitem toluidyny. Komórki tuczne różowo-fioletowe (barwienie metachromatyczne) a tło bladoniebieskie (barwienie ortochromatyczne). Nieliczne mastocyty degranulujące. Powiększenie 400 ×

mast cells were counted semi-automatically in 5 serial slides of stomach (corpus), duodenum and proximal colon made in each animal, in 10 consecutive visual fields under magnification 200 ×. The number of cells were then calculated for 10 area of view (AOV).

Data are expressed as mean and standard deviation (SD). Results were analysed by one-way analysis of variance (ANOVA), followed by LSD post-hoc test with Statistica 9.0 software package (StatSoft, Tulsa). Statistical significance was set at $p < 0.05$.

RESULTS

Mast cells in the gastrointestinal wall were identified by light microscopy as purple-red stained cells with multiple granules (Fig. 1). Some of those cells were fully or partially degranulated.

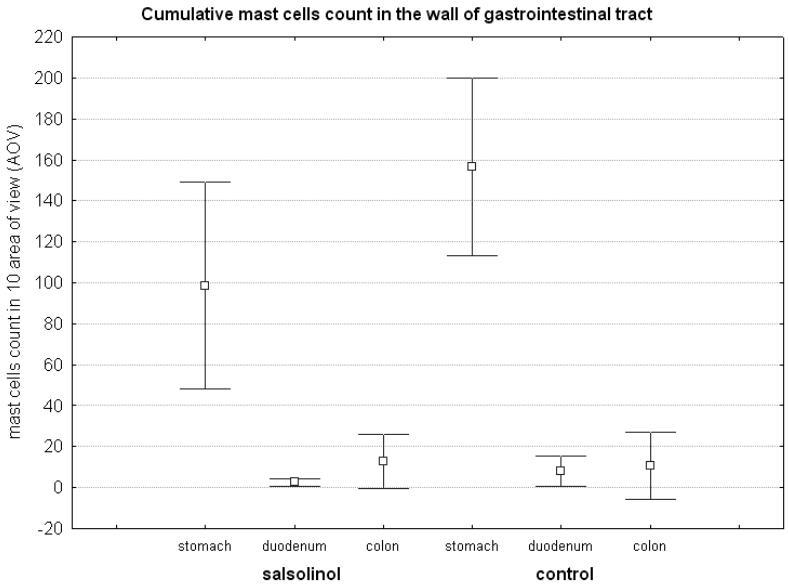

Fig. 2. Cumulative mast cells count in the stomach, duodenum and proximal colon in control and salsolinol treated rats (n = 8 for each group). Cells were counted in 10 area of view with magnification 200 × in toluidine blue stained specimens in mucosa, muscularis externa and serosa. Data are presented as mean and standard deviation

Ryc. 2. Całkowita liczba komórek tucznych w żołądku, dwunastnicy i proksymalnym odcinku jelita grubego w grupie kontrolnej oraz u szczurów po podaniu salsolinolu (n = 8 dla każdej z grup). Mastocyty liczono w 10 polach widzenia pod powiększeniem 200 × w preparatach barwionych błękitem toluidyny obejmujących całą grubość ściany. Wyniki przedstawiono jako średnie wraz z odchyleniem standardowym (SD)

The number of mast cells was decreased in the salsolinol-treated group compared to the control — in stomach 98.7 ± 53.3 vs. 156.7 ± 45.8, — in duodenum 2.6 ± 2.1 vs. 7.83 ± 7.8 and — in colon 12.8 ± 14 vs.10.7 ± 17.1 (salsolinol-treated group vs. control group) (Fig. 2).

The number of mast cells in the stomach muscularis externa was significantly decreased in the salsolinol-treated group compared to the control — 48.5 ± 41.8 vs. 119.0 ± 35.2. In duodenum 2.4 ± 2.3 vs. 6.3 ± 7.2 and in colon — 0.75 ± 1.5 vs. 3.1 ± 1.9 (salsolinol-treated group vs. control group) the number of mast cells in muscularis externa remained unchanged (Fig. 3).

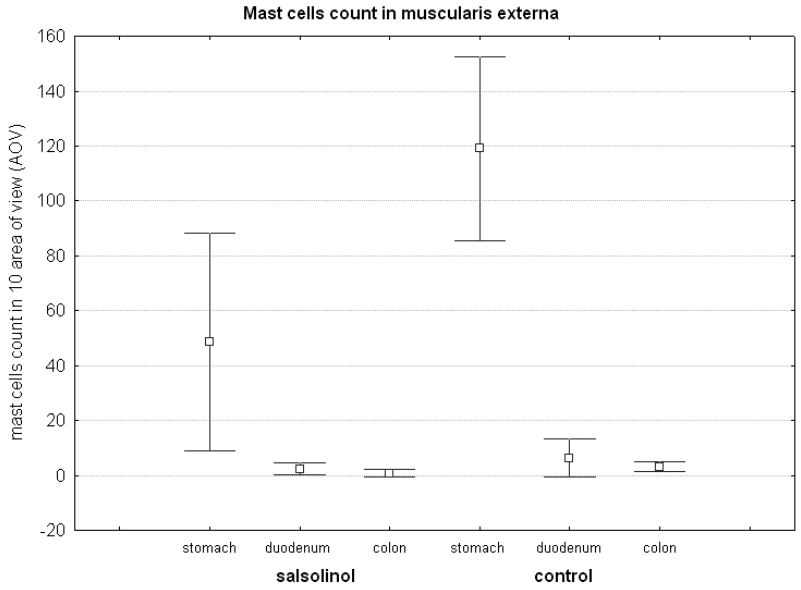

Fig. 3. Mast cells count in muscularis externa of the stomach, duodenum and proximal colon in control and salsolinol treated rats (n = 8 for each group). Cells were counted in 10 area of view with magnification 200 × in toluidine blue stained specimens. Data are presented as mean and standard deviation

Ryc. 3. Liczba komórek tucznych w błonie mięśniowej żołądka, dwunastnicy i proksymalnego odcinka jelita grubego w grupie kontrolnej oraz u szczurów po podaniu salsolinolu (n = 8 dla każdej z grup). Mastocyty liczono w 10 polach widzenia pod powiększeniem 200 × w preparatach barwionych błękitem toluidyny. Wyniki przedstawiono jako średnie wraz z odchyleniem standardowym (SD)

Surprisingly, the number of mast cells in the stomach mucosa was slightly but not significantly increased in salsolinol-treated group compared to control — 50.2 ± 16.1 vs. 37.7 ± 16.4 (Fig. 4). We did not find any changes in mast cells count in mucosa and muscularis externa in duodenum and proximal colon, because the small quantity of mast cells in these parts of gastrointestinal tract did not allowed to perform reliable statistical analysis.

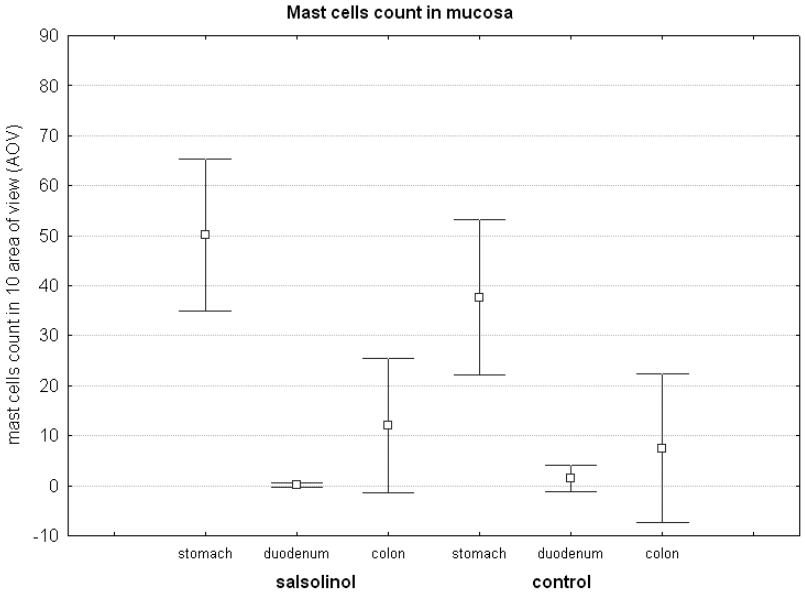

Fig. 4. Mast cells count in mucosa of the stomach, duodenum and proximal colon in control and salsolinol treated rats (n = 8 for each group). Cells were counted in 10 area of view with magnification 200 × in toluidine blue stained specimens. Data are presented as mean and standard deviation

Ryc. 4. Liczba komórek tucznych w błonie śluzowej żołądka, dwunastnicy i proksymalnego odcinka jelita grubego w grupie kontrolnej oraz u szczurów po podaniu salsolinolu (n = 8 dla każdej z grup). Mastocyty liczono w 10 polach widzenia pod powiększeniem 200 × w preparatach barwionych błękitem toluidyny. Wyniki przedstawiono jako średnie wraz z odchyleniem standardowym (SD)

The percentage of degranulated mast cells in the gastrointestinal wall was increased in the salsolinol-treated group compared to the control — in stomach 77.96 ± 10.7 vs. 17.34 ± 8.9, — in duodenum 50.0 ± 50.0 vs. 9.0 ± 17.5 and — in colon 61.36 ± 45.4 vs. 14.44 ± 37.9 (salsolinol-treated group vs. control group) (Fig. 5).

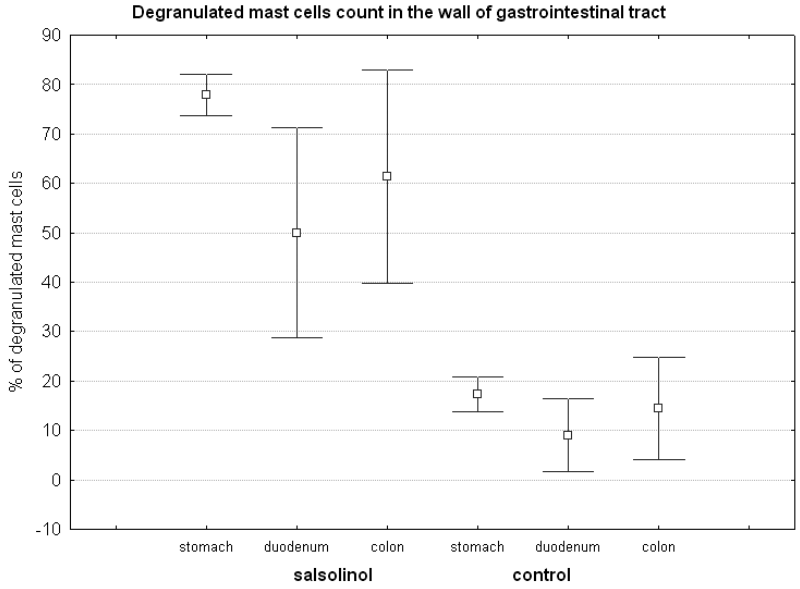

Fig. 5. Percentage of degranulated mast cells in the stomach, duodenum and proximal colon in control and salsolinol treated rats (n = 8 for each group). Cells were counted in 10 area of view with magnification 200 × in toluidine blue stained specimens in mucosa and muscularis externa. Data are presented as mean and standard deviation

Ryc. 5. Odsetek zdegranulowanych komórek tucznych w błonie śluzowej i błonie mięśniowej żołądka, dwunastnicy i proksymalnego odcinka jelita grubego u szczurów w grupie kontrolnej oraz po podaniu salsolinolu (n = 8 dla każdej z grup). Mastocyty liczono w 10 polach widzenia pod powiększeniem 200 × w preparatach barwionych błękitem toluidyny. Wyniki przedstawiono jako średnie wraz z odchyleniem standardowym (SD)

DISCUSSION

Mast cells develop from myeloid-cell progenitors under the influence of particular growth factors and are tissue cells typically located at important locations involved in host defence against the environment such as mucosal surfaces. The intestinal mucosa consists of around 2–3% of mast cells within the lamina propria in healthy individuals. In case of intestinal diseases, this amount can increase up to tenfold. For years, it has been generally accepted that mast cells are of relevance in gastrointestinal (GI) diseases such as food allergy, but recently, it became clear that mast cells participate in regulation of multiple tissue functions vital for gut homeostasis [10].

Animal models have proved valuable for studies of mast cells involvement in enteric immunoneural communication [3]. Infections with nematode para-

sites (Nippostrongylus brasiliensis) stimulate proliferation of intestinal mast cells in animal models. A second exposure to the infectious agent triggers release of mast cells mediators, which become paracrine signals to the ENS [11]. Receptors for mast cells mediators are expressed on the terminals of vagal and spinal afferents [12]. The vagus is thus responsible, at the subconscious level, for carrying and modulating physiological information from the gastrointestinal tract [13]. Long-term vagal nerve stimulation increases the number of mast cells in the gastrointestinal wall, suggesting close anatomical association between vagal fibres and enteric mast cells [14]. Stimulation of neurons in the brain stem by thyrotropin-releasing hormone evokes degranulation of mast cells in the rat small intestine adds another support for the brain-mast cell link [15]. The nerve — mast cell connection is significant because the gastrointestinal symptoms associated with mast cell degranulation are expected to be the same whether the mast cells are degranulated by antigen-antibody cross-linking in allergies or input from the ENS during stress conditions [3]. So, in fact, the communication is bi-directional, mast cells and nerves function as an integrated unit in the intestine. It appears that during inflammatory conditions, a positive feedback loop is established in which mast cells activate enteric nerves that further enhance mast cell activity, amplifying a secretory response to eliminate the luminal antigen responsible for the initial response [1]. And, there is evidence that the central nervous system modulates mast cell function via a vagal efferent pathway exclusively [5, 15]. The intestinal mucosa is uninterruptedly exposed to a load of antigens. The single-cell epithelial layer lining the gut lumen has the surface of around 300 m^2 and is the most important barrier between the external and internal environment. The intestinal barrier includes physical diffusion barriers, as well as enzymatic and immunological, all of which are under neurohormonal control. A breakdown of intestinal barrier function has been suggested as an etiologic factor in Crohn's disease, and in pathogenesis of viral and bacterial gastroenteritis, ulcerative colitis, and multiple organ dysfunction syndrome in patients with sepsis and trauma. And mast cells undoubtedly play an essential role in stress-related gut mucosal dysfunction by increasing epithelial permeability, for both small and large molecules [16]. Experiments with rodents have demonstrated that mast cell proteases are directly responsible for the increase of epithelial paracellular permeability and for redistributed expression of tight junctions during parasitic infection and stress. The fact that mast cells are in close contact with epithelial cells and nerve endings underline the hypothesis that mast cells are involved in regulating mucosal permeability and therefore intestinal barrier function, and can contribute to an ongoing inflammatory process. The increased mucosal permeability may lead to an enhanced influx of potentially threatening microbes into the intestinal tissue [10]. Mast cells, itself, by virtue of their location are potential targets for environmental agents with immunotoxic effects and once

activated, secrete numerous vasoactive, neurosensitizing and proinflammatory molecules. Mast cell-derived mediators can increase gut-blood and blood-brain barrier (BBB) permeability. Selective release of Vascular Endothelial Growth Factor, especially its vasodilatory isoform, may lead to BBB disruption, permitting brain inflammation. And selective release of IL-6 may also affect brain function and may influence hypothalamic-pituitary-adrenal axis [17].

Another neurotoxin, salsolinol, might represent a neuromodulator, which participates in the equilibrium of transmission of information at synapses composed of presynaptic neurons synthesizing dopamine as their primary neurotransmitter [18]. Salsolinol and its metabolites can be detected in many areas of the brain, rich in dopamine, especially in the striatum [19]. It occurs that only (R) enantiomer of salsolinol is present in the brain, however, both (R) and (S) enantiomers are found in human plasma and urine [20]. The enantiomer selective occurrence of salsolinol suggests that it might be endogenously synthesized in nerve bodies or synaptic terminals of dopamine neurons. Any direct evidence is still missing. But, it is known that glial cells are able to take up a wide range of neurotransmitters [21]. Salsolinol could lead to neurotoxicity by inhibition of mitochondrial complex II activity, increasing the formation of hydroxyl radicals or by initiation of apoptotic dopaminergic cell death [18].

Our results showed intestinal mucosal mast cells depletion in the gastrointestinal wall in the salsolinol-treated group in comparison with the control group. However at the same time the percentage of degranulated cells was raised in the salsolinol-treated group in comparison with the control group. We suggest then, it might be due to excessive mast cells degranulation caused by salsolinol. And once activated, mast cells might secrete a range of neurosensitizing and proinflammatory molecules, increasing gut-blood and blood-brain barrier.

Early involvement of the ENS is proposed in the pathogenesis of sporadic Parkinson's disease. Neuroactive substances are usually taken up at synapses, where they are frequently controlled by receptor-mediated endocytosis and transported to the cell body via the axon. The absence of a myelin sheath around axons of the first neurons in the potential chain of vulnerable projection neurons may facilitate entrance and damage by unknown pathogens. Most of the neurons located within the gastrointestinal wall, both intrinsic enteric neurons as well as extrinsic preganglionic parasympathetic and sympathetic fibres, lack such a protective barrier [7]. At the same time, the heterogeneity of pathological findings leading to a variety of alterations of GI motility underscore the need for appropriate animal models to investigate in depth the GI impairment in PD. At present, an ideal animal model is lacking [22]. Salsolinol and its active metabolites may well participate in the equilibrium of information transmission at synapses where dopamine is the primary neurotransmitter [20] but it remains

unclear if the loss of dopaminergic neurons in the gut determines inhibition of contractility and constipation [22].

The mast cells in the gastrointestinal tract have been found in close spatial contact with the regulatory cells of gastrointestinal motility: interstitial cells of Cajal (ICC) and myenteric neurons, suggesting their functional interaction. Because of the regulatory role of mast cells even the slight damage or change in activity of these cells may cause considerable disorder of the gut motility in PD patients [11, 23].

Mast cells serve in both innate and adaptive immune responses, and contribute to pathology of several disorders. However, many issues of mast cell biology still remains to be resolved, especially to what extent various environmental factors may influence their population. Can mast cells generate functionally distinct subsets, or have sufficient plasticity to develop distinct features on the basis of their responsiveness to local or systemic environmental signals? And how the understanding of their biology can be exploited clinically, to promote health and decrease prevalence of disease?

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WPŁYW SALSOLINOLU NA KOMÓRKI TUCZNE PRZEWODU POKARMOWEGO SZCZURA

Streszczenie

Liczne wyniki badań wskazują, że komórki tuczne (mastocyty) odgrywają ważną rolę przekaźnikową oraz pozostają w ścisłym kontakcie z komórkami regulatorowymi przewodu pokarmowego — komórkami Cajala oraz neuronami splotów śródmięśniowych. Ze względu na regulacyjną funkcję komórek tucznych nawet niewielkie ich uszkodzenie może przełożyć się na znaczące zaburzenia funkcji przewodu pokarmowego.

Pochodne katecholowe z grupy izochinoliny należą do grupy związków endogennych obecnych w mózgu ssaków. Ich głównym przedstawicielem jest salsolinol — 1-metylo-6,7-dihydroksy-1,2,3,4--tetrahydroizochinolina. Zaobserwowany u pacjentów z chorobą Parkinsona wzrost poziomu salsolinolu może być jedną z przyczyn zaburzeń funkcji przewodu pokarmowego u tych pacjentów.

Celem przeprowadzonych badań była ocena wpływu egzogennego salsolinolu na komórki tuczne w przewodzie pokarmowym szczura.

Szczury rasy Wistar (n = 8) otrzymywały dootrzewnowo salsolinol w dawce 50 mg/kg/dzień przez 3 tygodnie, a szczury grupy kontrolnej (n = 8) otrzymywały sól fizjologiczną. W ostatnim dniu eksperymentu szczury zgilotynowano i pobrano do badań fragmenty żołądka, dwunastnicy oraz jelita grubego. Ze skrawków parafinowych przygotowano preparaty, które wybarwiono błękitem toluidyny. Określano liczbę mastocytów oraz procent zdegranulowanych komórek tucznych w ścianie żołądka, dwunastnicy oraz części proksymalnej jelita grubego.

W grupie zwierząt otrzymujących salsolinol całkowita liczba komórek tucznych była mniejsza w porównaniu z grupą kontrolną we wszystkich odcinkach przewodu pokarmowego, a zwłaszcza w żołądku, natomiast odsetek komórek zdegranulowanych jest znamiennie wyższy w grupie szczurów poddanych działaniu salsolinolu.

Otrzymane wyniki wskazują na toksyczny wpływ salsolinolu na komórki tuczne we wszystkich odcinkach przewodu pokarmowego.

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